Angiotensin II Amplifies Arterial Contractile Response to Norepinephrine without Increasing Ca++ Influx: Role of Protein Kinase C[1]

DANIEL HENRION, ISMAIL LAHER, REGENT LAPORTE and JOHN A. BEVAN

Department of Pharmacology and Vermont Center for Vascular Research, University of Vermont, College of Medicine, Burlington, Vermont

Accepted for publication February 3, 1992

ABSTRACT

We investigated whether the enhanced contractile response to norepinephrine caused by a subthreshold concentration of angiotensin II was associated with an increased $^{45}Ca^{++}$ influx or net uptake. Rabbit facial artery segments were mounted isometrically to measure the $^{45}Ca^{++}$ influx and net uptake in response to norepinephrine. The contractile response to norepinephrine (3 μM) in the presence of angiotensin II (0.1 nM) was 149.5 ± 7.4% of control. This response amplification was not associated with changes in norepinephrine-induced $^{45}Ca^{++}$ influx or net uptake. Angiotensin II also potentiated the contractile response to caf-feine obtained in a Ca^{++}-free buffer containing ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetic acid (2 mM) to 148.0 ± 4.8% of control. In both cases, the amplification was prevented by pretreatment with either staurosporine (10 nM) or calphostin C (100 nM), two inhibitors of protein kinase C. We conclude that angiotensin II potentiation of norepinephrine-induced vascular tone occurs in the absence of changes in stimulated Ca^{++} entry. This potentiation may be due to an increase in intracellular sensitivity to Ca^{++}, possibly mediated by protein kinase C.

The potentiation by angiotensin II of the contractile response of vascular smooth muscle to sympathetic nerve stimulation and adrenoceptor activation is widely documented (Liao and Zimmerman, 1972; Day and Moore, 1976; Povolny et al., 1977; Duckles, 1981; Weber et al., 1985). For example, subthreshold concentrations of the vasoconstrictor angiotensin II potentiate the contractile response of arteries to norepinephrine (Van-houtte et al., 1981), clonidine (Laher et al., 1990), thrombin (Laher et al., 1990) and potassium (Day and Moore, 1976). Amplification of contractile responses of arteries is also caused by subthreshold concentrations of neuropeptide Y (Edvinsson et al., 1984; Andriantsitohaina and Stoclet, 1988), endothelin (Tabushi et al., 1989; Yang et al., 1990) and serotonin (Van Nueten, 1987; Szabo et al., 1991).

Although the mechanism(s) of angiotensin II potentiation of vascular responses is unknown, it appears to be a receptor-mediated phenomenon (Zeigler et al., 1986) requiring extracellular Ca^{++} (Weber et al., 1989), which occurs without the release of vasoactive factors from the endothelium (Weber et al., 1989). It seems most likely that this amplification results from changes in a common intracellular process regulating the contractile process because the vascular response to a number of contractile agents acting through different surface receptors is increased (Xiao and Rand, 1989). However, the possibility that the amplifying agents like angiotensin II partially depolarize vascular smooth muscle cells and/or increase the open-state probability of Ca^{++} channels in these cells (Nelson et al., 1988) cannot be discounted. A subthreshold dose of angiotensin II could bring the membrane potential closer to the threshold potential for norepinephrine-induced contraction. Thus, a subsequent application of norepinephrine would result in a greater contraction.

In this study, we report that angiotensin II amplifies norepinephrine-induced contraction without an associated augmentation of $^{45}Ca^{++}$ influx or net uptake. This amplification is prevented by the protein kinase C inhibitors staurosporine or calphostin C. Our results suggest that the intracellular sensitivity to Ca^{++} may be nonspecifically enhanced during contractile amplification in vascular smooth muscle.

Methods

Facial arteries were isolated from adult New Zealand male rabbits (2–4 kg) after anesthesia with pentobarbital (50 mg/kg, i.v. combined with heparin 1000 I.U./kg) and subsequent exsanguination. Ring segments, 5 mm in length, were mounted between two stainless steel wires in a 30-ml organ bath containing physiological salt solution (PSS) of the following composition (in mM): NaCl, 160; KCl, 4.6; $CaCl_2$, 1.5;

$MgSO_4$, 1.2; N-2-hydroxy-ethylpiperazine-N-2-ethylsulfonic acid, 5.0; glucose, 11.0. The pH of the PSS was adjusted to 7.4 with NaOH (1 M) and the solution was bubbled with 100% O_2. One wire was attached to a fixed support and the second wire was connected to a moveable holder supporting a tension transducer (Grass FT.03) so that isometric force measurements could be recorded on a physiograph (Grass SD90). Further details of the method used are described by Bevan and Osher (1972). The artery segments were allowed to recover for 60 min, during which time the PSS was replaced at 15-min intervals. After this recovery period, a 0.5-g preload resulting in stretch to optimum was applied to the artery and the stretched tissue was allowed to equilibrate for an additional 60 min.

Contractile response to norepinephrine and histamine. A concentration curve to norepinephrine was made by cumulative addition of norepinephrine to the PSS. After the maximal response to norepinephrine was obtained, the PSS was replaced several times until tissues returned to base-line tension. Norepinephrine (3 μM, corresponding approximately to a midrange response, the EC_{50} for norepinephrine being 3.15 μM in this preparation, see under "Results") was added to the PSS for 5 min and the contraction measured. This was repeated at 30-min intervals until the responses to successive additions became constant. Tissues were then pretreated for 10 min with angiotensin II (0.1 nM), and norepinephrine (3 μM) was added again. Control segments did not receive angiotensin II and responses to the final addition of norepinephrine (3 μM) are expressed as a percentage of the penultimate contractile response to norepinephrine in both groups (control and angiotensin II-treated). Some tissues were pretreated with the protein kinase C inhibitors staurosporine (10 nM, 30 min) or calphostin C (100 nM, 60 min) in addition to angiotensin II before the introduction of norepinephrine. In a separate series of experiments, histamine (0.3 μM corresponding to a midrange response) was added to the PSS for 5 min and the contraction measured. The protocol described for norepinephrine was applied to these experiments with histamine. After stabilization of the response, angiotensin II (0.1 nM) was added to the bath solution 10 min before histamine. In some experiments, calphostin C (100 nM) was added to the bath solution 60 min before the application of histamine (0.3 μM).

Contractile response to caffeine. After the contractile response to successive additions of norepinephrine (3 μM) was consistent and reproducible, arterial segments were then contracted with a maximally effective concentration of potassium (K^+, 80 mM). In such experiments, K^+-rich PSS was prepared by equimolar substitution by KCl for NaCl. Tissues were pretreated with prazosin (0.1 μM) and propranolol (1.0 μM) for 30 min before this addition of K^+ in order to block any adrenoceptor contribution to the induced contraction from the release of neuronal stores of norepinephrine. Once the response to K^+ reached a maximum, tissues were washed repeatedly with Ca^{++}-free PSS containing EGTA (2 mM) and allowed to equilibrate for 30 min. Preliminary experiments indicated that after this procedure, K^+ (80 mM) did not cause a contraction. Caffeine (5 mM) was then added and the resultant contraction measured. Pilot experiments indicated that the maximally effective dose of caffeine was 5 mM in the rabbit facial artery and produced a contraction corresponding to 12% of the maximal response to K^+ depolarization. In some tissues, angiotensin II (0.1 nM) was added during the last 10 min of the wash in Ca^{++}-free PSS containing EGTA.

In some other experiments, in addition to angiotensin II, staurosporine (10 nM) or calphostin C (100 nM) was present in the Ca^{++}-free PSS containing EGTA for 30 or 60 min (respectively) before the caffeine (5 mM) - induced contraction. In all cases, only one response to caffeine was elicited from each vessel segment.

Net $^{45}Ca^{++}$ uptake. Measurements of net uptake of $^{45}Ca^{++}$ were made at 37°C using the methods described by Meisheri et al. (1981). The net uptake of $^{45}Ca^{++}$ corresponds to a steady-state concentration resulting from a balance between $^{45}Ca^{++}$ influx and extrusion (van Breemen et al., 1985). After stabilization of the contractile response to norepinephrine (3 μM), arteries were placed in 30 ml of PSS containing $^{45}CaCl_2$ (0.67 μCi/ml) for 90 min. Tissues were then exposed to norep-

inephrine (3 μM) for 3 min, either in the absence or presence of angiotensin II (0.1 nM) added to the loading solution 10 min earlier. Resting values of net uptake of $^{45}Ca^{++}$ were determined by placing tissues in bubbled PSS containing $^{45}CaCl_2$ (0.67 μCi/mlμCi) for 93 min. In some experiments, arteries were incubated with $^{45}CaCl_2$ for 93 min and angiotensin II (0.1 nM) added during the last 10 min.

After exposure to $^{45}CaCl_2$ either with or without norepinephrine and/or angiotensin II, tissues were placed in 100 ml of ice-cold bubbled PSS for 45 min in order to remove the extracellularly bound $^{45}Ca^{++}$ (van Breemen et al., 1985). Artery segments were then blotted dry, weighed and incubated overnight in EDTA (5 mM) before counting in a liquid scintillation counter (Beckman LS 7800). Values of net uptake of $^{45}Ca^{++}$ are expressed as μmol/kg.

$^{45}Ca^{++}$ influx. During the maintained response of arteries to norepinephrine (3 μM), tissues were placed in PSS containing $^{45}Ca^{++}$ (0.67 μCi/ml) and norepinephrine (3 μM) for 90 sec. In some instances, angiotensin II (0.1 nM) was applied for 10 min before exposure to norepinephrine (3 μM) in $^{45}Ca^{++}$-PSS containing angiotensin II (0.1 nM) for 90 sec. This short period was selected to limit $^{45}Ca^{++}$ movements across the plasma membrane to mainly influx (Aaronson and van Breemen, 1981; van Breemen et al., 1985). Resting values of $^{45}Ca^{++}$ influx were determined by placing arteries in PSS containing $^{45}Ca^{++}$ (0.67 μCi/ml) for 90 sec. The effect of angiotensin II alone on $^{45}Ca^{++}$ influx was measured by first pretreating artery segments with angiotensin II (0.1 nM) for 10 min before placing tissues in $^{45}Ca^{++}$ PSS containing angiotensin II (0.1 nM).

Finally, artery segments were placed in 100 ml of ice-cold bubbled PSS for 45 min, blotted dry, weighed and incubated overnight in EDTA (5 mM) before counting in a liquid scintillation counter (Beckman LS 7800). Values of $^{45}Ca^{++}$ influx are expressed as μmol/kg/min.

Statistical analysis. Results are expressed as the mean ± S.E.M. Comparisons between groups were made using the Student's t test, either paired or unpaired, or a one-way analysis of variance followed by a Student-Newman-Keuls test or an appropriate Student's t test (paired or unpaired), when significant. A probability level of $P < .05$ is considered significant.

Drugs. Angiotensin II, caffeine and norepinephrine were purchased from Sigma Chemical Co. (St. Louis, MO). Staurosporine was purchased from Kyowa Hakko USA Inc. (New York, NY). Calphostin C was purchased from Kamiya Biomedical Co. (Thousand Oaks, CA). All other reagents were of analytical grade. Stock solutions of norepinephrine contained hydrochloric acid (0.01 M). Staurosporine and calphostin C were dissolved in dimethylsulfoxide (1 mg/ml). $^{45}CaCl_2$ (23.1 μCi/mg) was purchased from New England Nuclear (Boston, MA).

Results

Interaction of angiotensin II with norepinephrine and with histamine on rabbit facial artery. Norepinephrine produced concentration-dependent contractions of the rabbit facial artery with an EC_{50} of 3.15 ± 0.26 μM ($n = 12$). The maximal response produced by norepinephrine (0.1 mM) was 4.03 ± 0.41 g. Angiotensin II (0.1 nM) pretreatment potentiated the contraction to a midrange concentration of norepinephrine (3 μM) to 149 ± 7.4% of control (table 1; fig. 1). After pretreatment with either calphostin C or staurosporine, the response to norepinephrine (3 μM) in the presence of angiotensin II was not significantly different from control responses elicited before its addition (table 1; fig. 1). The contractile response to norepinephrine (3 μM) of arteries pretreated with staurosporine alone or calphostin C alone were not significantly different from their controls (table 1). Histamine produced concentration-dependent contractions of the rabbit facial artery (EC_{50} of 0.48 ± 0.15 μM, and maximal response of 3.84 ± 0.77 g, $n = 6$). Angiotensin II (0.1 nM) pretreatment amplified the histamine (0.3 μM) induced contraction to 130.0 ± 18.4% of control (table

TABLE 1

Potentiation by angiotensin II of norepinephrine and histamine contractile response of the rabbit facial artery and effect of the protein kinase C inhibitors staurosporine and calphostin C

Results are mean ± S.E. of six to eight experiments.

Treatment	Control	Pretreatment with Angiotensin II (0.1 nM)	Potentiation (%)
Norepinephrine (3 μM) contraction (g)			
None	2.09 ± 0.17	2.98 ± 0.10*	142.7 ± 12.8
Calphostin C (100 nM)	2.05 ± 0.20	2.11 ± 0.25	103.1 ± 8.2
None	2.10 ± 0.30	3.20 ± 0.40*	156.3 ± 8.1
Staurosporine (10 nM)	2.10 ± 0.15	1.92 ± 0.20	91.5 ± 6.6
Histamine (0.3 μM) contraction (g)			
None	1.80 ± 0.34	2.34 ± 0.22*	130.0 ± 18.4
Calphostin C (100 nM)	1.74 ± 0.27	1.72 ± 0.38	98.9 ± 15.0

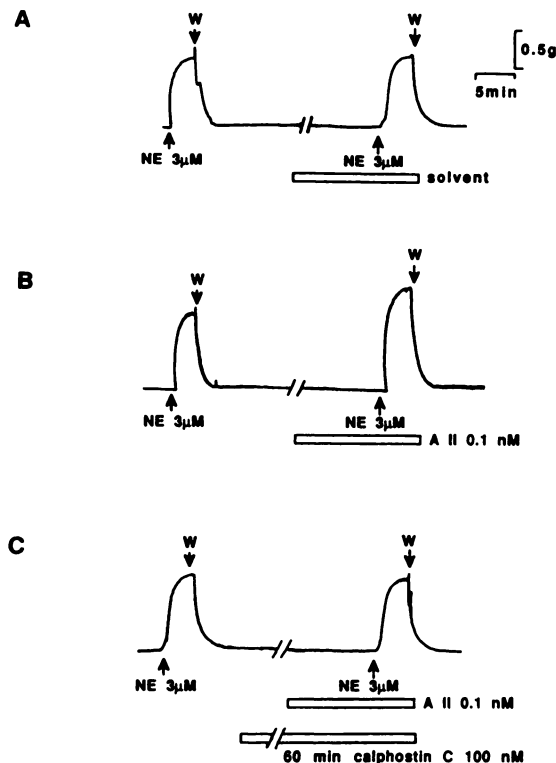* $P < .05$, significantly different from control (no inhibitor), paired Student's t test.

Fig. 1. Amplification of the contractile response to norepinephrine (NE: 3 μM) of the rabbit facial artery by angiotensin II (AII: 0.1 nM) and effect of the protein kinase C inhibitor calphostin C (100 nM). A) Control experiment: 30 μl of PSS (solvent of AII) was added to the bath solution 10 min before the second NE (3 μM) contractile response. B) AII (0.1 nM) was added to the bath solution 10 min before the second NE (3 μM) contractile response. C) Calphostin C (100 μM) was added 60 min and AII (0.1 nM) 10 min before the second NE (3 μM) contractile response.

1). Calphostin C (100 nM) pretreatment abolished the potentiation effect of angiotensin II (0.1 μM) (table 1).

Contraction to caffeine. The absolute force development (g) and the augmentation induced by angiotensin II of the responses to caffeine (5 mM) are summarized in table 2 and a typical recording is shown in figure 2. Caffeine caused transient contractile responses in arteries incubated in a Ca^{++}-free PSS containing EGTA (2 mM). After pretreatment with angiotensin II, this transient contractile response to caffeine was significantly increased to 148 ± 5.8%. The contractile response to caffeine of arterial segments treated with either staurosporine or calphostin C was not significantly altered by angiotensin II (table 2).

$^{45}Ca^{++}$ **fluxes.** The resting $^{45}Ca^{++}$ influx and net uptake and that in response to norepinephrine, angiotensin II or a combination of these are summarized in figure 3. Both $^{45}Ca^{++}$ parameters followed the same pattern. Exposure to norepinephrine for 3 min increased significantly the $^{45}Ca^{++}$ fluxes over resting values. This increase in fluxes was not influenced by prior exposure to angiotensin II. Similarly, exposure of tissues to angiotensin II alone had no effect on the $^{45}Ca^{++}$ fluxes.

Discussion

The present study provides evidence for a postsynaptic intracellular mechanism for the augmentation of vascular tone due to vasoconstrictor agents. The main findings are that: 1) angiotensin II increases the sensitivity of arterial segments to norepinephrine and histamine; 2) the enhanced contractile response to norepinephrine induced by angiotensin II is not associated with an increase in either norepinephrine-induced influx or net uptake of extracellular $^{45}Ca^{++}$; 3) in the absence of extracellular Ca^{++}, the contractile response to caffeine is amplified by angiotensin II; and 4) staurosporine and calphostin C, both inhibitors of protein kinase C, attenuate the ability of angiotensin II to amplify the contractile response to either norepinephrine or caffeine, and calphostin C attenuates the potentiation by angiotensin II of the histamine-induced contraction. Our results suggest that an increased entry of extracellular Ca^{++} is not essential for the augmented contractile response to norepinephrine and histamine induced by angiotensin II.

There are a number of studies on the interaction of angiotensin II with norepinephrine in isolated segments of rabbit femoral arteries. Using several criteria, Purdy and Weber (1988) concluded that pretreatment with angiotensin II does not unmask a subpopulation of latent postsynaptic adrenoceptors during the vasoconstriction caused by norepinephrine. Weber et al. (1989) determined that the extent of augmentation of the norepinephrine-induced tone by angiotensin II is not dependent on the release of vasoactive factors from endothelial cells. More recently, Laher et al. (1990) suggested that tonic modulation of protein kinase C activity increases the sensitivity of intracellular contractile mechanisms associated with Ca^{++}-dependent vasoconstriction. This provides a nonspecific mechanism for response amplification.

Although the amplifying effect of angiotensin II on the vasoconstrictor action of adrenergic agonists is well established, the specific mechanisms involved are unknown. Angiotensin II receptor stimulation is essential (Zeigler et al., 1986), as is the

TABLE 2

Potentiation by angiotensin II of caffeine contractile response of the rabbit facial artery and effect of the protein kinase C inhibitors staurosporine and calphostin C

Results are mean ± S.E.; number of experiments enclosed in parentheses.

Treatment	Caffeine (5 mM) Contraction (g)		
	Control	Pretreatment with Angiotensin II (0.1 nM)	Potentiation (%)
None	0.50 ± 0.05 (13)	0.74 ± 0.06 (13)*	148.0 ± 5.8
Staurosporine (10 nM)	0.54 ± 0.06 (9)	0.51 ± 0.07 (9)	94.4 ± 10.1
Calphostin C (100 nM)	0.52 ± 0.02 (8)	0.55 ± 0.05 (8)	105.8 ± 6.9

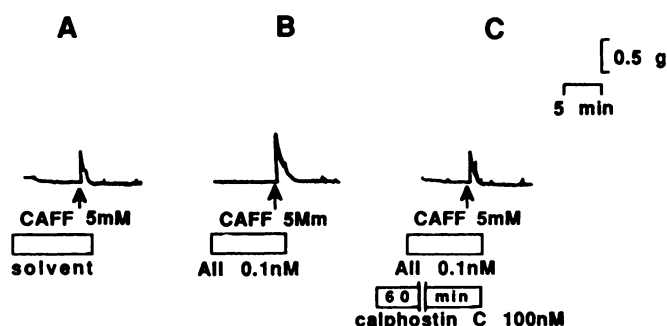* $P < .05$, significantly different from control, unpaired Student's t test.

Fig. 2. Amplification of the contractile response of the rabbit facial artery to caffeine (CAFF: 5 mM) by angiotensin II (AII) and effect of the protein kinase C inhibitor calphostin C (100 nM). A) Control experiment: 30 μl PSS (solvent of AII) was added to the bath solution 20 min before caffeine (5 mM). B) AII (0.1 nM) was added to the bath solution 10 min before caffeine. C) Calphostin C (100 nM) was added 60 min and AII (0.1 nM) 10 min before caffeine (5 mM).

presence of extracellular Ca^{++} (Weber et al., 1989). One possible mechanism is either a direct increase of Ca^{++} influx or an increase of the open-state probability of Ca^{++} channels during activation by norepinephrine (see Nelson et al., 1990 for a detailed discussion of the effects of neurotransmitters on vascular Ca^{++} channels). The most direct means whereby angiotensin II could affect Ca^{++} entry is by depolarizing the vascular smooth muscle cell (Nelson et al., 1988, 1990). A change in membrane potential could explain a change in sensitivity of the vascular smooth muscle to agonist. To verify such a possibility, measurements of the membrane potential and/or of calcium channel activity would be helpful. However, to consider such a scheme, two criteria would have to be satisfied.

First, measurable increases in either $^{45}Ca^{++}$ influx or net uptake reflecting initial calcium entry should occur. As mentioned under "Methods," our measurement of $^{45}Ca^{++}$ net uptake reflects the steady-state bidirectional movement of Ca^{++} across the plasma membrane (i.e., the balance between influx and extrusion) (van Breemen et al., 1985). A subthreshold concentration of angiotensin II (0.1 nM) for contraction of the rabbit facial artery was used in the present study. This concentration did not increase either influx or net uptake of extracellular $^{45}Ca^{++}$. In addition, angiotensin II pretreatment did not cause an additional increase in the norepinephrine-induced influx or net uptake of extracellular $^{45}Ca^{++}$. Yet, under comparable conditions, norepinephrine contractile responses were significantly augmented by the peptide (table 1). We cannot, however, exclude an increase in Ca^{++} influx due to angiotensin II too small to be distinguished by these techniques. Nevertheless, in glomerulosa cells, angiotensin II has been reported to increase the activity of intracellular Ca^{++}-sensitive processes without

causing a net change in cytosolic free Ca^{++} concentration (Kojima et al., 1984).

Second, if angiotensin II causes its effect by influencing plasmalemmal Ca^{++} channel behavior so as to promote Ca^{++} entry, then its amplifying effect should be absent in a medium free of extracellular Ca^{++}. We tested this possibility by examining the contractile responses to caffeine obtained in a Ca^{++}-free medium containing EGTA. Under these circumstances, the transient contractile response to caffeine is most likely due to the release of Ca^{++} from internal stores, presumably the sarcoplasmic reticulum (Leijten and van Breemen, 1984; Karaki et al., 1987). However, angiotensin II still augmented caffeine-evoked contractions (table 2). This finding suggests that a mechanism not directly related to increased availability of extracellularly derived Ca^{++} underlies the amplification.

Our hypothesis, supported by previous data (Laher et al., 1990) as well as by the experiments described in this article, is that the increased activity or sensitivity of an intracellular Ca^{++}-sensitive process must underlie amplification. For this to be the case, an effect on a process common to most agonists would be necessary. It has been reported that angiotensin II pretreatment augments the contractile responses to norepinephrine (Vanhoutte et al., 1981), serotonin (van Nueten, 1983), histamine (Day and Moore, 1976), thrombin (Laher et al., 1990), Bay K 8644 (Laher et al., 1990), potassium (Day and Moore, 1976) and caffeine (this study). In the rabbit facial artery, both the histamine (0.3 μM)- and the norepinephrine-induced contraction were amplified by angiotensin II (0.1 nM). The potentiation by angiotensin II of the contraction induced by two different agonists confirms the validity of the use of the rabbit facial artery. We have proposed elsewhere that low concentrations of angiotensin II increase the activity of protein kinase C so as to increase the effectiveness of a given concentration of cytosolic free Ca^{++} (Laher et al., 1990).

Our results are compatible with reports indicating that in cultured vascular smooth muscle cells, angiotensin II causes a sustained increase in diacylglycerol formation (Griendling et al., 1989). The subsequent activation of protein kinase C leads to phosphorylation of a number of intracellular proteins (Griendling et al., 1986) such that the sensitivity of Ca^{++}-dependent processes is increased (Laher and Bevan, 1989; Nishimura and van Breemen, 1989; Ruzycky and Morgan, 1989). Therefore, it is conceivable that upon stimulation with angiotensin II, the contractile response to agents causing both extracellular Ca^{++} entry and intracellular Ca^{++} release (e.g., norepinephrine and histamine) or only intracellular Ca^{++} release (e.g., caffeine) would be augmented. Consistent with this are observations that staurosporine and calphostin C, putative inhibitors of protein kinase C, separately cancel the amplifying effects of angiotensin
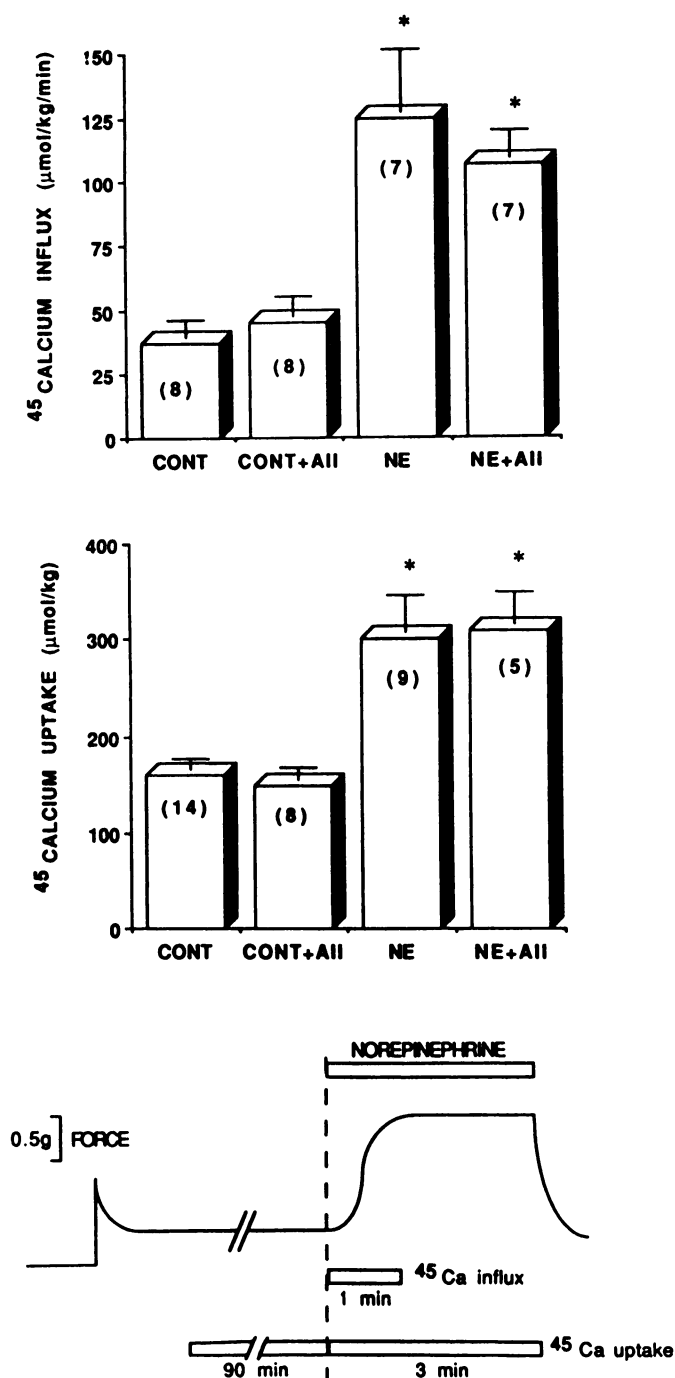
Fig. 3. Effects of norepinephrine (NE, 3 μM) on $^{45}Ca^{++}$ influx (upper panel) and $^{45}Ca^{++}$ uptake (medium panel) in the rabbit facial artery and effect of a pretreatment with angiotensin II (0.1 nM) on the control values (CONT + AII) of both $^{45}Ca^{++}$ fluxes and on the $^{45}Ca^{++}$ flux values with norepinephrine (NE + AII). In control experiments (CONT), norepinephrine was not added to the physiological salt solution. Lower panel: schematic representation of the $^{45}Ca^{++}$ influx and uptake measurements. Figures in parentheses refer to the number of experiments. *P < .05, significantly different from the corresponding control, one-way analysis of variance.

II. The concentrations of staurosporine (10 nM) and calphostin C (100 nM) used in our study do not inhibit myosin light chain kinase activity, as determined by their inability to inhibit K$^+$-evoked contractions in the rabbit facial vein (unpublished observations), rabbit femoral artery (Laher *et al.*, 1990), rabbit basilar artery (Germann *et al.*, 1991), rat midcerebral artery (Osol *et al.*, 1991) and rat mesenteric arterioles (Osol *et al.*,

1991). Nevertheless, although contractile function data do not indicate an inhibition of myosin light chain kinase by staurosporine, we cannot exclude this because its K_i for myosin light chain kinase inhibition has been estimated at 20 nM (Buchholz *et al.*, 1991), which is close to the concentration used in the present study (10 nM). Calphostin C, compared to staurosporine, is about 20 times less inhibitory for protein kinase C (IC$_{50}$ = 0.05 μM *vs.* 0.0027 μM; Tamaoki *et al.*, 1986; Kobayashi *et al.*, 1989), but at least 2500 times less inhibitory for myosin light chain kinase (IC$_{50}$ > 5.00 μM, Kamiya Biomedical Co. unpublished results, *vs.* 0.0019 μM; Nakanishi *et al.*, 1991). This may possibly be related to the fact that staurosporine inhibits protein kinase C at its catalytic domain, which is conserved throughout the protein kinase family, whereas calphostin C inhibits protein kinase C at its specific phorbol ester binding site in the regulatory domain (Tamaoki and Nakano, 1990).

Additional support for our hypothesis is provided by other studies which have demonstrated that the exogenous activation of protein kinase C (*e.g.*, by phorbol esters) amplifies vasoconstriction due to serotonin in the rabbit aorta (Consigny, 1989), to alpha-2 adrenoceptor stimulation in the rat saphenous vein (Cheung, 1988) and to potassium depolarization in the rat saphenous vein and tail artery (Cheung, 1988). Activation of protein kinase C by phorbol esters has also been shown to sensitize arterial contraction to Ca^{++}-ionophore A23187 and to Ca^{++} channel agonist Bay K 8644 (Wei and Triggle, 1986; Purdy and Weber, 1988).

In conclusion, we have shown that angiotensin II potentiates both the norepinephrine and the histamine-induced contraction. Moreover, in the absence of extracellular calcium, the caffeine-induced contraction was amplified by angiotensin II and the amplification of the norepinephrine-induced contraction did not involve a higher level of either $^{45}Ca^{++}$ influx or uptake. The potentiation phenomenon is probably mediated through the activation of protein kinase C, as it was abolished by the protein kinase C inhibitors staurosporine and calphostin C, and does not require an increase in Ca^{++} influx.

References

AARONSON, P. AND VAN BREEMEN, C.: Effects of sodium gradient manipulation upon cellular ^{45}Ca fluxes and cellular sodium in the guinea-pig taenia coli. J. Physiol. 319: 443–461, 1981.

ANDRIANTSITOHAINA, R. AND STOCLET, J. C.: Potentiation by neuropeptide Y or vasoconstriction in rat resistance arteries. Br. J. Pharmacol. 95: 219–228, 1988.

BEVAN, J. A. AND OSHER, J. V.: A direct method for recording tension changes in the wall of small blood vessels in vitro. Agents Actions 2: 257–260, 1972.

BUCHHOLZ, R. A., DUNDORE, R. L., CUMISKEY, W. R., HARRIS, A. R. AND SILVER, P. J.: Protein kinase C inhibitors and blood pressure control in spontaneously hypertensive rats. Hypertension 17: 91–100, 1991.

CHEUNG, D. W.: Phorbol 12,13-dibutyrate potentiates alpha-2-adrenoceptor-mediated contraction in the rat saphenous vein. Eur. J. Pharmacol. 158: 145–148, 1988.

CONSIGNY, P. M.: Phorbol amplification of serotonin-induced arterial contractions is endothelium dependent. Am. J. Physiol. 257: H1174–H1179, 1989.

DAY, M. D. AND MOORE, A. F.: Interaction of angiotensin II with noradrenaline and other spasmogens on rabbit isolates aortic strips. Archs int. Pharmacodyn. Ther. 219: 29–44, 1976.

DUCKLES, S. P.: Angiotensin II potentiates responses of the rabbit basilar artery to adrenergic nerve stimulation. Life Sci. 28: 635–640, 1981.

EDVINSSON, L., EKBLAD, E., HAKANSON, R. AND WAHLESTEDT, C.: Neuropeptide Y potentiates the effect of various constrictor agents on rabbit blood vessels. Br. J. Pharmacol. 83: 519–525, 1984.

GERMANN, P., LAHER, I. AND BEVAN, J. A.: Platelets augment rabbit cerebral artery constriction by activating protein kinase C. Stroke 22: 1532–1540, 1991.

GRIENDLING, K. K., RITTENHOUSE, S. E., BROCK, T. A., EKSTEIN, L. S., GIMBRONE, M. A. AND ALEXANDER, R. W.: Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. J. Biol. Chem. 261: 5901–5906, 1986.

GRIENDLING, K. K., TSUDA, T., BERK, B. AND ALEXANDER, R. W.: Angiotensin

II stimulation of vascular smooth muscle. J. Cardiovasc. Pharmacol. **14**: S27–S33, 1989.

HENRION, D., LAPORTE, R., LAHER, I. AND BEVAN, J. A.: Angiotensin II potentiates norepinephrine and caffeine-induced contraction by activation of protein kinase C. Circulation **84**: II-625, 1991.

KARAKI, H., AHN, H. Y. AND URAKAWA, N.: Caffeine-induced contraction in vascular smooth muscle. Arch. Int. Pharmacodyn. **285**: 60–71, 1987.

KOBAYASHI, E., NAKANO, H., MORIMOTO, M. AND TAMAOKI, T.: Calphostin C (UCN-1028C), a novel microbial compound, is a highly potent and specific inhibitor of protein kinase C. Biochem. Biophys. Res. Commun. **159**: 548–553, 1989.

KOJIMA, I., KOJIMA, K., KREUTLER, D. AND RASMUSSEN, H.: The temporal integration of the aldosterone secretory response to angiotensin occurs via two intracellular pathways. J. Biol. Chem. **259**: 14448–14457, 1984.

LAHER, I. AND BEVAN, J. A.: Staurosporine, a protein kinase C inhibitor, attenuates Ca^{2+}-dependent stretch-induced vascular tone. Biochem. Biophys. Res. Commun. **158**: 58–62, 1989.

LAHER, I., THOMPSON, L. P. AND GAGNE, L.: Protein kinase C as a modulator of response amplification in vascular smooth muscle. Blood Vessels **27**: 333–340, 1990.

LEIJTEN, P. A. A. AND VAN BREEMEN, C.: The effects of caffeine on the noradrenaline-sensitive calcium store in rabbit aorta. J. Physiol. **357**: 327–339, 1984.

LIAO, J. C. AND ZIMMERMAN, B. G.: Response to sympathetic stimulation during angiotensin tachyphylaxis in dog's paw. Archs int. Pharmacodyn. Ther. **197**: 362–369, 1972.

MEISHERI, K. D., HWANG, O. AND VAN BREEMEN, C.: Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. J. Membr. Biol. **59**: 19–25, 1981.

NAKANISHI, S., KASE, H. AND MATSUDA, Y.: Assay of myosin light chain kinase activity by high-performance liquid chromatography using a synthetic peptide as substrate. Anal. Biochem. **195**: 313–318, 1991.

NELSON, M. T., STANDEN, N. B., BRAYDEN, J. E. AND WORLEY, J. F.: Noradrenaline contracts arteries by activating voltage dependent calcium channels. Nature (Lond.) **336**: 382–385, 1988.

NELSON, M. T., PATLAK, J. B., WORLEY, J. F. AND STANDEN, N. B.: Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am. J. Physiol. **259**: C3–C18, 1990.

NISHIMURA, J. AND VAN BREEMEN, C.: Direct regulation of smooth muscle contractile elements by second messengers. Biochem. Biophys. Res. Commun. **163**: 929–935, 1989.

OSOL, G., LAHER, I. AND CIPOLLA, M.: Protein kinase C modulates basal myogenic tone in resistance arteries from the cerebral circulation. Circ. Res. **68**: 359–367, 1991.

POVOLNY, K. M., JUNG, R. W., KRAFT, E. AND ZIMMERMAN, B. G.: Adrenergic potentiation by angiotensin II in isolated canine cutaneous arteries: effect of bathing media and calcium. Blood Vessels **14**: 105–115, 1977.

PURDY, R. E. AND WEBER, M. A.: Angiotensin II amplification of α-adrenergic vasoconstriction: role of receptor reserve. Circ. Res. **63**: 748–757, 1988.

RUZYCKY, A. L. AND MORGAN, K. G.: Involvement of the protein kinase C system

in calcium-force relationships in ferret aorta. Br. J. Pharmacol. **97**: 391–400, 1989.

SZABO, C., HARDEBO, J. E. AND OWMAN, C.: An amplifying effect of exogenous and neurally stored 5-hydroxytryptamine on the neurogenic contraction in rat tail artery. Br. J. Pharmacol. **102**: 401–407, 1991.

TABUSHI, Y., NAKAMARU, M., RAKUGI, H., NAGANO, M. AND OGIHARA, T.: Endothelin enhances adrenergic vasoconstriction in perfused rat mesenteric arteries. Biochem. Biophys. Res. Commun. **159**: 1304–1308, 1989.

TAMAOKI, T. AND NAKANO, H.: Potent and specific inhibitors of protein kinase C of microbial origin. Biotechnology **8**: 732–735, 1990.

TAMAOKI, T., NOMOTO, H., TAKAHASHI, I., KATO, Y., MORIMOTO, M. AND TOMITA, F.: Staurosporine, a potent inhibitor of phospholipid/Ca^{2+}-dependent protein kinase. Biochem. Biophys. Res. Commun. **135**: 397–402, 1986.

VAN BREEMEN, C., HWANG, K., LOUTZENHISER, R., LUKEMAN, S. AND YAMAMOTO, H.: Ca^{2+} entry into vascular smooth muscle. In Bayer-Symposium IX—Cardiovascular Effects of Dihydropyridine-Type Calcium Antagonists and Agonists, ed. by A. Fleckenstein, C. van Breemen, R. Gross and F. Hoffmeister, pp. 58–71, Springer-Verlag, Berlin, 1985.

VAN NUETEN, J. M.: 5-Hydroxytryptamine and precapillary vessels. Fed. Proc. **42**: 223–227, 1983.

VAN NUETEN, J. M.: Interactions between serotonin and other vasoconstrictor agonists in vascular tissues. In Neuronal Messengers in Vascular Function, ed. by A. Nobin, C. Owman and B. Arneklo-Nobin, pp. 385–397, Elsevier Science Publishers, New York, 1987.

VANHOUTTE, P. M., VERBEUREN, T. J. AND WEBB, R. C.: Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. Physiol. Rev. **61**: 151–247, 1981.

WEBER, M. A., DRAYER, J. I. M., PURDY, R. E., FRANKFORT, P. P. AND RICCI, B. A.: Enhancement of the pressor response to norepinephrine by angiotensin in the conscious rabbit. Life Sci. **36**: 1897–1907, 1985.

WEBER, M. A., PURDY, R. E., STUPECKY, G. L. AND PRINS, B. A.: Augmentation of sympathomimetic arterial contraction by angiotensin II: a novel mechanism. J. Vasc. Med. Biol. **1**: 7–12, 1989.

WEI, X. Y. AND TRIGGLE, D. J.: Ca^{2+} channel ligand sensitive responses of the phorbol ester 12-o-tetradecanoylphorbol 13-acetate in vascular smooth muscle. Can. J. Physiol. Pharmacol. **64**: 1489–1496, 1986.

XIAO, X. H. AND RAND, M. J.: α_2-Adrenoreceptor agonists enhance vasoconstrictor responses to α_1-adrenoreceptor agonists in the rat tail artery by increasing the influx of Ca^{2+}. Br. J. Pharmacol. **98**: 1032–1038, 1989.

YANG, Z., RICHARD, V., VON SEGESSER, L., BAUER, E., STULZ, P., TURINA, M. AND LUSCHER, T. F.: Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries—a new mechanism for vasospasm? Circulation **82**: 188–195, 1990.

ZEIGLER, D. W., JOHNSON, J. A., KOIVUNEN, D. G., SIRIPAISARNPIPAT, S., FOWLER, W. L., JR., DOSTAL, D. E. AND PAYNE, C. G.: Angiotensin receptors and pressor hyperresponsiveness in renal prehypertensive rabbits. Am. J. Physiol. **251**: H196–H204, 1986.